





hours per day, 5 days per week, for 2, 4, 8, or 13 weeks.

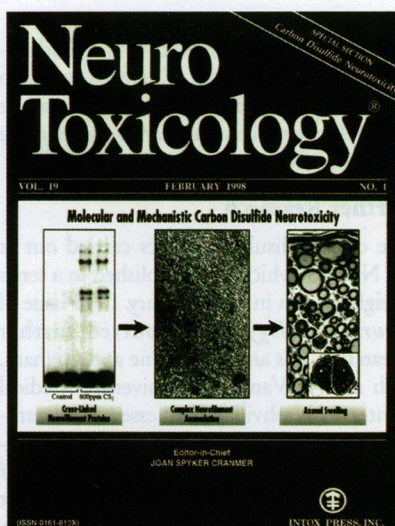
An important consideration in designing the studies was the need to correlate the inhaled CS<sub>2</sub> dose and the neurobehavioral, neurophysiological, and neuropathological effects. To do this, it was essential that all the endpoints be obtained on all of the animals. These endpoints included pharmacokinetic changes in blood CS<sub>2</sub> levels and urinary metabolites, blood and spinal cord biomarkers of exposure and effect, neurotoxicity alterations in axon-Schwann cell interactions, pathology of the peripheral nerve and spinal cord, nerve conduction velocity (NCV), compound nerve action potential (CNAP), and behavioral assessments using a Functional Observational Battery (FOB). Immediately following exposure on the day prior to the penultimate exposure, urine was collected for metabolic analysis. During and after the next inhalation exposure, blood was collected for analysis of CS<sub>2</sub> and metabolites. Following the last exposure, rats were examined by FOB and then assessed for electrophysiological function. Next, 5 rats per sex per dose group were evaluated for the presence of CS<sub>2</sub> protein-protein cross-linking in the red blood cells and the neurofilaments of the spinal cord. Segments of the sciatic nerve were collected and analyzed for alterations in the low-affinity nerve growth factor receptor (NGF-R) mRNA. Four rats per sex per dose group were selected for the morphologic evaluations.

## Results

One of the prerequisites for characterizing the toxicity of any compound is to understand how the compound is taken up by the body and either retained or eliminated. The concentration and duration of exposure and the number of previous days of exposure to the compound all can affect the amount that is actually retained. To evaluate the relationship between measures of the inhalation exposure to CS<sub>2</sub> and the toxicologic response and biomarkers of exposure, the research team performed three separate studies characterizing CS<sub>2</sub> kinetics in the test animals.

A single exposure inhalation study was conducted to investigate the uptake and elimination kinetics of CS<sub>2</sub>. A single-exposure intravenous study was also conducted to estimate the volume of distribution and total systemic clearance. Finally, a 13-week inhalation study involving repeated exposures characterized the plateau of CS<sub>2</sub> blood levels and 2-thiothiazolidine-4-carboxylic acid (TTCA) excretion.

The studies found that, at the concentrations tested (50, 500, and 800 ppm),



**The results are in.** The award-winning CS<sub>2</sub> studies were published in a special section in the February 1998 issue of *Neurotoxicology*.

there is not a linear dose-response relationship between the amount of CS<sub>2</sub> inhaled and either blood CS<sub>2</sub> concentration or urinary TTCA excretion. Both blood CS<sub>2</sub> and urinary TTCA became saturated at these higher levels. Thus, these measures appear to be useful only as indicators of exposure to relatively low levels of CS<sub>2</sub> exposure and short exposure time frames.

Using samples obtained from the NIEHS collaboration, it was demonstrated that covalent cross-linking of neurofilament proteins was a direct effect of CS<sub>2</sub> contributing to the formation of neurofilamentous axonal swellings, a lesion characteristic of CS<sub>2</sub> neurotoxicity. It was also shown that cross-linking of neurofilament proteins was positively correlated to CS<sub>2</sub>-mediated covalent cross-linking of spectrin, a red blood cell membrane protein. A linear dose-response relationship was observed for these two biochemical events, suggesting that populations exposed to CS<sub>2</sub> could be evaluated through periodic blood sampling, and that the quantity of spectrin cross-linking could be used to identify people in danger of developing neurotoxicity. Hemoglobin has a similar biological life to that of erythrocyte spectrin, and is easier to isolate and analyze. As part of the NIEHS research, scientists sought to evaluate modified hemoglobin as a potential dosimeter for quantifying exposure to CS<sub>2</sub>.

The study found that hemoglobin modification does, indeed, possess several advantages over spectrin cross-linking as a biomarker of effect for CS<sub>2</sub> exposure. Modification of hemoglobin can be detected at earlier time points than spectrin dimer formation. Analysis of hemoglobin requires drawing far less blood than that of spectrin and can be performed with more

direct and rapid methods. Collectively, the findings suggest that alterations in the alpha chain of hemoglobin may provide a sensitive neurotoxic biomarker of effect for CS<sub>2</sub> with the potential to provide mechanistically relevant assessments of subchronic exposures, a tool to help identify susceptible individuals, and a means to examine possible effects occurring at presently acceptable levels of CS<sub>2</sub> exposure.

Researchers also examined the potential of using mRNA expression of NGF-R as an early indicator of peripheral nervous system damage. One of the effects of chronic CS<sub>2</sub> exposure is the retraction of myelin, the fatty substance that ensheathes nerve fibers. Previous research had shown that mRNA levels are markedly upregulated in the sciatic nerve during demyelination. Upregulation also occurs in various subdegenerative axonopathy models where there is axonal atrophy. These findings suggested that mRNA upregulation could be a useful biomarker for subtle perturbations in normal axon-Schwann cell interactions. To further test this hypothesis, the team examined NGF-R mRNA expression in sciatic nerves of rats exposed to CS<sub>2</sub>.

The study revealed that NGF-R mRNA expression does increase in a dose- and time-dependent manner. Morphologic alterations in the sciatic nerve were not apparent, even at the highest dosages with the longest exposure times. Thus, upregulation of NGF-R mRNA is an indicator of subtle alterations in the normal axon-Schwann cell relationship and does provide a sensitive measure of CS<sub>2</sub> neurotoxicity. Researchers state that the assay of this marker may also be useful as a rapid and sensitive general screen for other compounds that are potentially toxic to the peripheral nervous system.

One of the most widely reported findings in people and animals exposed to CS<sub>2</sub> is neurotoxicity in the central and peripheral nervous system. Yet few studies have fully examined the morphologic progression, biology, and mechanism of CS<sub>2</sub>-induced neurotoxicity. Therefore, the researchers conducted a study to examine the progression and dose response of CS<sub>2</sub> distal axonopathy by light and electron microscopy and in teased nerve fiber preparations. They then correlated these observations with other biologic and mechanistic findings using inhalation studies.

The study revealed that both behavioral changes and biochemical effects, such as cross-linking of hemoglobin and neurofilament proteins, and increases in NGF-R mRNA expression occur prior to axonal swelling. The study illustrates that the detection of neurotoxic effects prior to



morphologic changes can be used to discern potential neurotoxicity and mechanisms of toxicity.

A frequently cited functional change following CS<sub>2</sub> exposure is a decrease in NCV. The researchers hypothesized that alterations in peripheral nerve function produced by CS<sub>2</sub> exposure would be reflected in changes in CNAP and/or NCV. Using electrophysiological testing and microscopic examination of the ventral caudal tail nerve, researchers quantified concentration- and time-related changes in peripheral nerve electrophysiology produced by subchronic exposure to CS<sub>2</sub>. This study revealed that exposures to 500 ppm or 800 ppm CS<sub>2</sub> for 8–13 weeks produced some minor changes in NCV and CNAP recorded from the ventral caudal tail nerves of experimental animals. The biological basis for the changes in CNAP produced by CS<sub>2</sub> is currently under investigation.

A final study measured behavioral changes in test animals using a FOB. Neuromuscular deficits, including gait alterations and decreased hindlimb and forelimb grip strength, were detected in the test animals after as little as two weeks' exposure to 800 ppm CS<sub>2</sub>. These changes were closely related to CS<sub>2</sub> concentration and exposure duration. Other effects, observed mostly at 13 weeks, included

decreased responsiveness to a visual stimulus and mild tremors. Correlations with other endpoints in the project demonstrate that behavioral changes can be a sensitive indicator of CS<sub>2</sub> neurotoxicity.

### Further Research

The carbon disulfide studies carried out at the NIEHS, which were published in a series of eight papers in the February 1998 issue of *Neurotoxicology*, have spurred further research in this area. Valentine and Graham, both now at Vanderbilt University Medical Center in Nashville, Tennessee, used samples from the NIEHS studies in a study published in the January 1997 issue of *Toxicology and Applied Pharmacology* that demonstrated the sensitivity of spectrin cross-linking for detecting inhalation exposures to CS<sub>2</sub>, and provided evidence for covalent cross-linking of neurofilament proteins as a mechanism for CS<sub>2</sub>-induced axonal neurofilamentous swellings. They also used the biomarkers developed in the CS<sub>2</sub> inhalation study in a second study, published in the February 1998 issue of the same journal, to quantify the amount of CS<sub>2</sub> liberated by diethyldithiocarbamate *in vivo*. A third study, published in the May 1998 issue of *Chemical Research in Toxicology*, established the formation of CS<sub>2</sub>-mediated thiourea protein cross-links on spectrin *in vivo*.

Valentine and Graham now intend to investigate the utility of CS<sub>2</sub>-mediated protein modifications for monitoring high-risk human populations. "The information derived from the human studies should indicate how useful our biomarkers are for developing intervention strategies for identifying and removing individuals from neurotoxic levels of CS<sub>2</sub> or compounds that release CS<sub>2</sub> prior to their developing neurological deficits," says Valentine.

The EPA, meanwhile, anticipates using the data from the CS<sub>2</sub> studies for a series of actions defined under the Clean Air Act Amendments of 1990. "These studies will be vital to the EPA for development and refinement of mechanistically based quantitative human health risk assessments for chemicals listed in the Clean Air Act," says Gary Foureman, a health scientist with the EPA's National Center for Environmental Assessment in Research Triangle Park. "These risk assessments, in turn, are used for such measures as the listing and delisting of hazardous air pollutants, setting of emission standards through Maximum Achievable Control Technology, and providing technical assistance through air toxics clearinghouses such as the Air Risk Information Support Center."

John Manuel

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